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Research review paper

Natural products from resurrection plants: Potential for medical applications

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ABSTRACT

Resurrection species are a group of land plants that can tolerate extreme desiccation of their vegetative tissues during harsh drought stress, and still quickly – often within hours – regain normal physiological and metabolic functions following rehydration. At the molecular level, this desiccation tolerance is attributed to basal cellular mechanisms including the constitutive expression of stress-associated genes and high levels of protective metabolites present already in the absence of stress, as well as to transcriptome and metabolome reconfigurations rapidly occurring during the initial phases of drought stress. Parts of this response are conferred by unique metabolites, including a diverse array of sugars, phenolic compounds, and polyols, some of which accumulate to high concentrations within the plant cell. In addition to drought stress, these metabolites are proposed to contribute to the protection against other abiotic stresses and to an increased oxidative stress tolerance. Recently, extracts of resurrection species and particular secondary metabolites therein were reported to display biological activities of importance to medicine, with e.g. antibacterial, anticancer, antifungal, and antiviral activities, rendering them possible candidates for the development of novel drug substances as well as for cosmetics. Herein, we provide an overview of the metabolite composition of resurrection species, summarize the latest reports related to the use of natural products from resurrection plants, and outline their potential for medical applications.

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Introduction

Resurrection species are a unique group of land plants that can tolerate desiccation of their vegetative tissues to air-dried state (to just 5%

relative water content, RWC) and resume normal physiological and metabolic activities after rehydration (Dinakar and Bartels, 2013; Gechev et al., 2012). Resurrection species are more common in bryophytes, relatively rare in pteridophytes and angiosperms, and absent in gymnosperms (Gaff and Oliver, 2013; Porembski, 2011). In total, resurrection plants represent more than 1300 different species, including about 300 angiosperms (Porembski, 2011). They display considerable geographic and habitat diversity. Most of them are herbaceous plants which inhabit deserts or temperate areas with extended periods of drought (Dinakar et al., 2012; Gechev et al., 2012). However, some of them (like the European resurrection plants *Ramonda serbica* and *Haberlea rhodopensis*) can endure freezing winters, and one of them, *Lindernia brevidens*, was recently discovered in the tropical rainforests of Africa, where humidity is constantly high (Phillips et al., 2008). It has to be noted that the number of resurrection plants is likely to grow as more studies discover new desiccation-tolerant species.

To adapt to extreme dehydration, resurrection plants have developed unique molecular mechanisms to protect themselves against desiccation-induced damage. These mechanisms, summarized in the recent review of Dinakar and Bartels (2013), are constitutive (expression of stress-protective genes and high abundance of protective metabolites) as well as inducible (swift transcriptome and metabolome reconfigurations occurring upon the sensing of drought stress). The remarkable geographical and habitat diversity of these species has further contributed to the diverse array of genes and metabolites which they utilize for stress protection and environmental adaptation.

Although the primary interest in resurrection species has been fueled by their ability to withstand desiccation and the potential to use them as a source for gene discovery (Gechev et al., 2013; Rodriguez et al., 2010; Yobi et al., 2012; Yobi et al., 2013), the unique metabolites of several resurrection species have recently attracted much attention with respect to their potential uses in biotechnology and medicine. For example, the predominant polyphenol 3,4,5 tri-O-galloylquinic acid in the South African resurrection species *Myrothamnus flabellifolia* has been shown to inhibit M-MLV and HIV-1 reverse transcriptases (Kamng'ona et al., 2011). Myconoside, a glycoside abundantly present in extracts of *H. rhodopensis*, can strongly stimulate antioxidant skin defenses and extracellular matrix protein synthesis (Dell'Acqua and Schweikert, 2012). Amentoflavone, isolated from *Selaginella tamariscina*, has strong anticancer/pro-apoptotic, antibacterial, and antifungal activities (Cheng et al., 2008; Gao et al., 2007; Woo et al., 2005). These and other bioactive features of resurrection plant metabolites are reviewed in this article with a particular focus placed on potential biomedical applications.

Overview of primary and secondary metabolites of resurrection species

The primary metabolites of resurrection species, as in all species, primarily serve to ensure basic physiological functions. However, some of the metabolites are additionally utilized as osmoprotectors against dehydration-induced stress. Comprehensive metabolome profiling has been performed for several resurrection species, including the monocot *Sporobolus stapfianus*, the dicot *H. rhodopensis*, and the spike moss *Selaginella lepidophylla* (Dinakar and Bartels, 2013; Gechev et al., 2013; Oliver et al., 2011a; Yobi et al., 2012; Yobi et al., 2013).

Sugar metabolism plays a paramount role in stress protection in plants. In desiccation-tolerant *S. lepidophylla*, some sugars such as sucrose, trehalose, and several monosaccharides are highly abundant, in contrast to its desiccation-sensitive sister species *Selaginella moellendorffii* (Yobi et al., 2012; Yobi et al., 2013). The basic levels of several sugars, including sucrose, raffinose, melibiose, and trehalose, are also very high in *H. rhodopensis* in comparison with other species like *Arabidopsis thaliana* or *Thellungiella halophila* (Benina et al., 2013). Sucrose accumulation is observed in most resurrection species during dehydration (Benina et al., 2013; Djilianov et al., 2011; Gechev et al.,

2013; Peters et al., 2007; Rakić et al., 2014; Yobi et al., 2012). Raffinose is another abundant sugar in most of the resurrection species and, like sucrose, can act as an osmoprotector (Peters et al., 2007). Additionally, raffinose and galactinol were suggested to protect against cellular damage caused by oxidative stress (Nishizawa et al., 2008). Some resurrection species contain less studied or even unique sugars. *Craterostigma plantagineum*, for example, accumulates large amounts of the 8-carbon sugar octulose, which is used as a carbohydrate reserve during dehydration (Bianchi et al., 1991; Norwood et al., 2000), while *H. rhodopensis* can accumulate verbascose, a constituent together with stachyose of the raffinose family of oligosaccharides (Gechev et al., 2013).

Resurrection species have abundant amounts of different sugar alcohols and sugar acids, which together with the sugars may collectively alleviate the consequences of dehydration by stabilizing proteins and other macromolecules, and protecting them from reactive oxygen species (ROS)-induced damage (Gechev et al., 2013; Oliver et al., 2011a; Yobi et al., 2012; Yobi et al., 2013). The basal levels of threonate, erythronate, and glycerate are much higher in *H. rhodopensis* than in *A. thaliana* or *T. halophila* (Benina et al., 2013). Resurrection species may also utilize di-carboxylic acids and various amino acids as an additional tool to alleviate dehydration. Nitrogen-rich and γ -glutamyl amino acids, citrulline, and nucleotide catabolism products increase in desiccated *S. lepidophylla* (Yobi et al., 2013).

Lipid metabolism can also change during dehydration and subsequent rehydration. While most lipids were produced constitutively in *S. lepidophylla*, choline phosphate accumulated during dehydration, suggesting a role in membrane hydration and stabilization (Yobi et al., 2013). On the other hand, several polyunsaturated fatty acids were found at higher levels in unstressed plants (Yobi et al., 2013). Like sugars, lipids may play multiple roles: as signaling molecules, as an energy source (especially after sugars are consumed), and as protectors against desiccation-induced damage (Beckett et al., 2012; Gasulla et al., 2013).

In contrast to primary metabolites, much less is known about secondary metabolites present in resurrection plants. Secondary metabolites are chemically very diverse in exhibiting many different biological functions. Although much progress has been made on elucidating their structure, function, and biosynthesis in the past decade, still many questions related to the biosynthetic pathways and their regulation remain to be explored. Furthermore, it is believed that we currently know only a small fraction of the rich diversity of secondary metabolites in the plant kingdom which has been estimated at about 200,000 compounds (Dixon and Sumner, 2003; Yonekura-Sakakibara and Saito, 2009). Resurrection plants contribute to this diversity, as evidenced by the presence of a wide range of unique compounds. So far, the structures of only a fraction of these metabolites have been resolved, a fact that is greatly complicated by the lack of commercially available reference compounds for secondary metabolism (Fernie, 2007); and we know even less about their biological functions in plants. In general, resurrection species utilize their secondary metabolites not only for protection against dehydration but also against other stresses such as UV-light and herbivore attack, thus gaining advantage over competitor species within particular ecological niches.

In several studies, many secondary metabolites that belong to different classes were identified in *Anastatica hierochuntica*. Among others, these included anastatins A and B, apigenin, luteolin, caffeoyl- and dicaffeoylquinic acids, (+)-dehydrodiconiferyl alcohol, 3,4-dihydroxybenzoic acid, eriodictyol, hierochins A and B, kaempferol, luteolin, quercetin, and silybins A and B (Al Gamdi et al., 2011; Nakashima et al., 2010; Yoshikawa et al., 2003).

Boea hygrometrica (Bunge) R. Br. (Gesneriaceae) is a resurrection plant distributed widely from the tropics to the northern temperate regions of East Asia (Mitra et al., 2013). It contains C-glycosylflavones and phenolic acids (5,7,3'-tetrahydroxy-6-methoxy-8-C-[β -D-xylopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranosyl flavone, *p*-hydroxy phenethyl alcohol, 3,4-dihydroxy phenethyl

alcohol, apocynin, ferulic acid, 1'-O- β -D-(3,4-dihydroxy)-ethyl-6'-O-*trans*-caffeoyl- β -D-apiofuranosyl-(1 \rightarrow 3')-glucopyranoside, gentisic acid) (Feng et al., 2011; Li et al., 2011).

A few secondary metabolites were also identified in *H. rhodopensis*, including hispidulin-8-C-(2"-O-syringoyl)- β -glucopyranoside, hispidulin 8-C-(6-O-acetyl- β -glucopyranoside), hispidulin 8-C-(6-O-acetyl-2-O-syringoyl- β -glucopyranoside), paucifloside, and myconoside (Ebrahimi et al., 2011).

Secondary metabolites have been more thoroughly studied in the medicinally used resurrection species *Myrothamnus flabellifolia* and *Myrothamnus moschatus*. There are similarities, but also differences, in the chemical profiles of the two species, as well as variation between populations from different regions (Nicoletti et al., 2012; Randrianarivo et al., 2013). Forty volatiles accounted for 98.6% of the essential oil in samples of *M. moschatus* (Nicoletti et al., 2012). The most abundant were *trans*-pinocarveol, pinocarpone, β -pinene, β -selinene, and perillyl acetate. In *M. flabellifolia*, *trans*-pinocarveol, pinocarpone, and β -selinene were also among the most abundant volatiles, along with α -pinene, limonene, and a few other terpenoids (Nicoletti et al., 2012).

In *S. stapfianus*, a number of terpenes and phenolic compounds have been identified including, among others, squalene, campesterol, quinate, sinapate, and caffeate (Oliver et al., 2011a). Several phenolic compounds and alkaloids have also been identified in *S. lepidophylla*, including apigenin, caniferyl alcohol, naringenin, sinapyl alcohol, and vanillate (Yobi et al., 2013). More attention to secondary metabolites was paid to the medicinal resurrection plant *Selaginella tamariscina*. Newly identified compounds in this species include amentoflavone (proapoptotic and tumoricidal, as well as antibacterial and antifungal activities), heveaflavone, hinokiflavone, isocryptomerin (antifungal activity), selaginellins A and B, 7"-O-methylamentoflavone, and others (Cheng et al., 2008; Gao et al., 2007; Woo et al., 2005).

It is clear that considerably more research is required for the identification and analysis of the mode of action of secondary metabolites, since in most cases they are the compounds which exhibit strong biological activities. They may be interesting enough for the development of novel drug substances (as such or as lead compounds).

Metabolites from resurrection species with potential medical applications

Most resurrection species are so far virtually unexplored with respect to their primary and secondary metabolic complements. Furthermore, from the limited number of resurrection plants with well-studied metabolomes (reviewed by Dinakar and Bartels, 2013), there are only few examples of well-characterized metabolites with biological activities. In many cases, the reported biological activities are of crude total extracts in which the compound that is presumably responsible for the observed activity is mixed with other compounds that are co-extracted (Ahn et al., 2006; Austin, 2004; Cano and Volpato, 2004; Dell'Acqua and Schweikert, 2012; Georgieva et al., 2013; Gescher et al., 2011; Mishra et al., 2011; Nicoletti et al., 2012; Nicoletti et al., 2012; Robles-Zepeda et al., 2011; Sah et al., 2005; Yang et al., 2013a, 2013b). Data on the exact composition of such extracts are often missing. The available information is usually limited to the type of extract (water, ethanol–water, etc.). In most of the cases, little or no information is given on the changes in metabolite abundances related to the hydration status of the plant (which can be a very important factor in the resurrection species). Nevertheless, the few examples written in bold below show the great potential of resurrection plants as a source of valuable bioactive compounds for use in medicine and cosmetics, or as lead for drug development. Given the discovery of new resurrection plants and the growing number of desiccation tolerant species with characterized metabolite compositions, more examples are to be expected in the near future and we envisage that some of them will most likely find their way to pharmaceutical applications.

Anastatica hierochuntica L. (Rose of Jericho or Jerusalem Resurrection Plant; Brassicaceae) is a desert resurrection plant distributed in North Africa (Sahara) and parts of Asia (Fig. 1) (Al Gamdi et al., 2011). Extracts of *A. hierochuntica* contain many flavonoids, glucosinolates, terpenes, and other secondary compounds such as apigenin, caffeoyl- and dicaffeoylquinic acids, 3,4-dihydroxybenzoic acid, glucoiberin, glucocheirolin, isovitexin, kaempferol-7-glucoside, luteolin, quercetin, silybins A and B, isosilybins A and B, and others (Al Gamdi et al., 2011; Nakashima et al., 2010; Yoshikawa et al., 2003). Some of these compounds (such as apigenin, luteolin, and quercetin, Fig. 2) were previously identified in other species and have long been recognized for their medicinal properties (Agarwal et al., 2013; Loguercio and Festi, 2011; Sak, 2014). For example, the widely distributed quercetin (one of the main dietary flavonoids, found in many plant species) is a potent antioxidant. Quercetin has been shown to inhibit the growth of various cancer cells (Sak, 2014) and its ingestion can inhibit platelet aggregation in human, thus reducing the risk of cardiovascular diseases (Hubbard et al., 2006). The antioxidant silybin is a well-known and clinically applied hepatoprotector (from *Silybum marianum*, milk thistle) and some of its derivatives showed prominent anti-cancer activity in vitro (Agarwal et al., 2013; Loguercio and Festi, 2011). Two flavonoids isolated from *A. hierochuntica*, anastatins A and B, exhibited hepatoprotective effect by reducing cytotoxicity of D-galactosamine on primary cultured murine hepatocytes (Fig. 2, Table 1) (Yoshikawa et al., 2003). The effect of anastatins was stronger than the commercially available hepatoprotector silybin. Among the compounds isolated from the whole plant of *A. hierochuntica*, isosilybins A and B, luteolin, quercetin, (+)-balanophonin, and 3,4-dihydroxybenzaldehyde inhibited melanogenesis in murine B16 melanoma 4A5 cells (IC₅₀ values of 10–17 μ M) to a much greater extent than the well-known tyrosinase inhibitor arbutin (Nakashima et al., 2010). Tyrosinase is an oxidase important for melanin production; hence its control is of relevance to medicine and cosmetics. Although only quercetin was shown to have a substantial inhibitory effect on enzyme activity (measured with mushroom tyrosinase), silybin B and isosilybins A and B inhibited the expression of genes encoding tyrosinase and tyrosinase-related proteins involved in melanin synthesis (Nakashima et al., 2010).

In addition to the antioxidant activities, antimicrobial activities were also reported for *A. hierochuntica* extracts (Daur, 2012). The activity against Gram-positive bacteria was higher than against Gram-negative bacteria (Mohamed et al., 2010). Administration of aqueous extracts of *A. hierochuntica* to normal and streptozotocin-induced diabetic rats revealed a hypoglycemic effect. It also caused improvement in tissue injury caused by streptozotocin (Rahmy and El-Ridi, 2002). Methanolic extracts of *A. hierochuntica* were recently shown to possess immunomodulatory effects in mice after oral administration, relating to IgG levels, phagocytosis and adenosine deaminase activity (Abdulfattah, 2013).

In Arabian folk medicine, *A. hierochuntica* is applied near birth, where it is soaked in water and drunk to avoid birthing pain. Further, the plant is traditionally used as analgesic, emmenagogue, hepatoprotectant, and anti-epileptic (Duke et al., 2010). Despite these proclaimed medicinal properties, there is little scientific evidence for the therapeutic effect in humans. A warning has been given against the use of this plant by pregnant women, until proven safe (Salah and Machado, 2012). In Malaysia, the use of *A. hierochuntica* by pregnant women is very popular because it contains many minerals that play a role in the maintenance of human health and it is believed to facilitate smooth delivery (Abdulfattah, 2013; Sooi and Keng, 2013).

Haberlea rhodopensis Friv. (Gesneriaceae) is a perennial dicot resurrection plant endemic to Europe's Balkan Peninsula (Gechev et al., 2013). It has attracted considerable attention recently as a model species to study desiccation tolerance and because of the antioxidant properties of its extracts (Dell'Acqua and Schweikert, 2012; Gechev et al., 2013; Kondeva-Burdina et al., 2013). A *H. rhodopensis* extract rich in the caffeoyl phenylethanoid glycoside

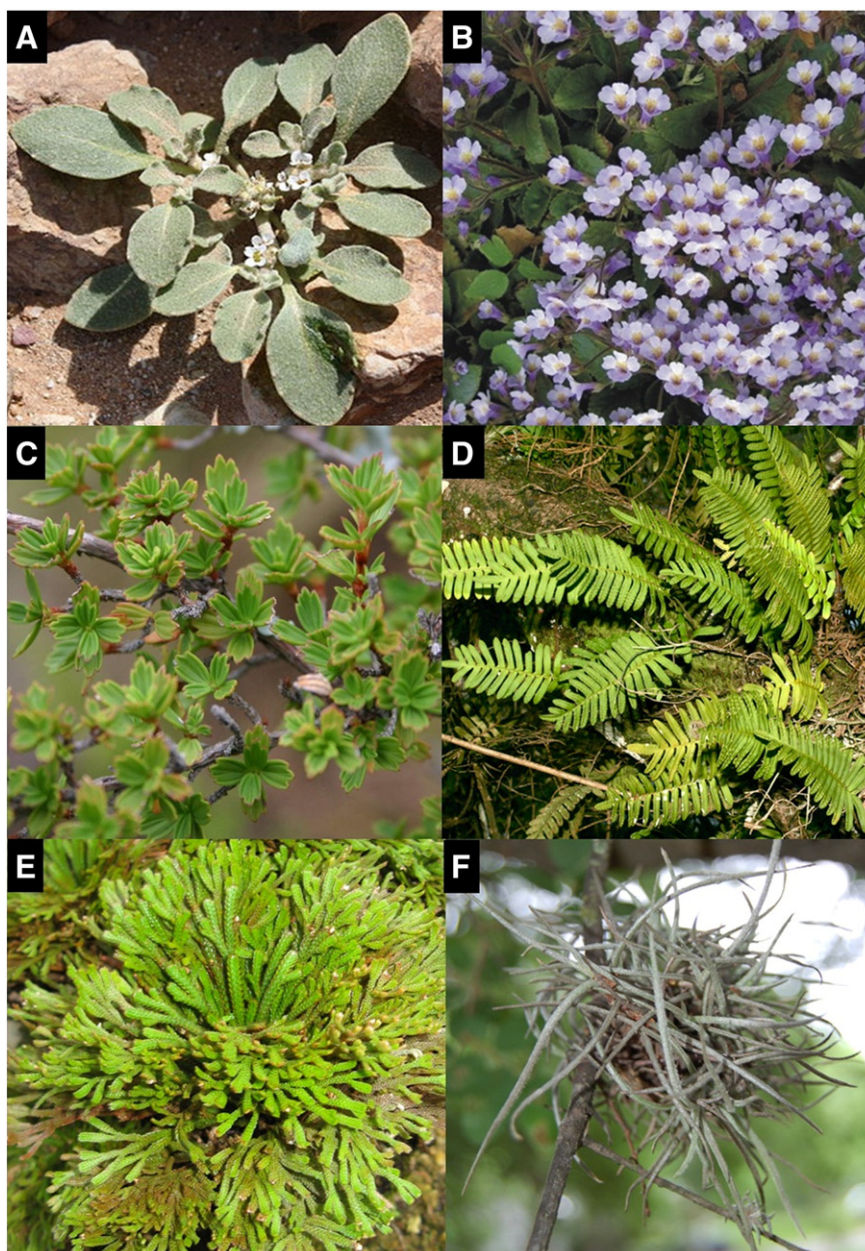


Fig. 1. Resurrection plants reported to have medicinal properties. A, *Anastatica hierochuntica*; B, *Haberlea rhodopensis*; C, *Myrothamnus flabellifolius*; D, *Polypodium polypodioides*; E, *Selaginella tamariscina*; and F, *Tillandsia recurvata*. Images A, C, D, E, and F were obtained from Wikimedia Commons under the GNU Free Documentation Licence (http://en.wikipedia.org/wiki/GNU_Free_Documentation_License). Image B is from our own collection.

myconoside increased mRNA synthesis of collagen and elastin genes in human dermal fibroblasts stressed with H_2O_2 (Dell'Acqua and Schweikert, 2012). Furthermore, the *Haberlea* extracts protected against UV-induced dermis oxidation and increased skin elasticity of human volunteers (Dell'Acqua and Schweikert, 2012). Based on these findings, it was suggested that an extract of *H. rhodopensis* can be used for anti-aging treatments, protecting the skin from oxidation, increasing skin elasticity and enhancing skin radiance (Dell'Acqua and Schweikert, 2012). For these reasons, *H. rhodopensis* has been used in anti-wrinkle cosmetic products (Elle and Kressaty, 2009).

It was shown that methanolic leaf extracts of *H. rhodopensis* have a strong antioxidant effect by reducing H_2O_2 -generated oxidative stress in both, non-neoplastic and prostate cancer cells. In the non-malignant cell line HEK 293 it had an apoptosis-protective and cell death-reducing effect when the cells were pre-treated before H_2O_2 -induced oxidative stress. NF κ B was activated in p53^{+/+} cells and

suppressed in p53^{-/-} cells (Hayrabyan et al., 2013). Additionally, extracts of *H. rhodopensis* protected rabbit blood cells from γ -radiation-induced DNA damage (chromosome aberrations) and oxidative stress (accumulation of malondialdehyde) (Georgieva et al., 2013). The decreased radiation-induced DNA damage and decreased oxidative stress in *H. rhodopensis*-treated samples was concomitant with increased antioxidant activities of catalases and superoxide dismutases (Georgieva et al., 2013).

The South African shrub *Myrothamnus flabellifolius* (Sonder) Welw. (Myrothamnaceae) is one of the best studied resurrection plants in terms of secondary metabolites with medicinal properties (Moore et al., 2007). It is a widely used plant in traditional African medicine. Its uses include the treatment of chest complaints (smoke of burning leaves), and wounds (in ointments for topical application), and to treat cough, influenza, mastitis, backaches, kidney disorders, hemorrhoids, abdominal pains, scurvy, halitosis and gingivitis (in the form of

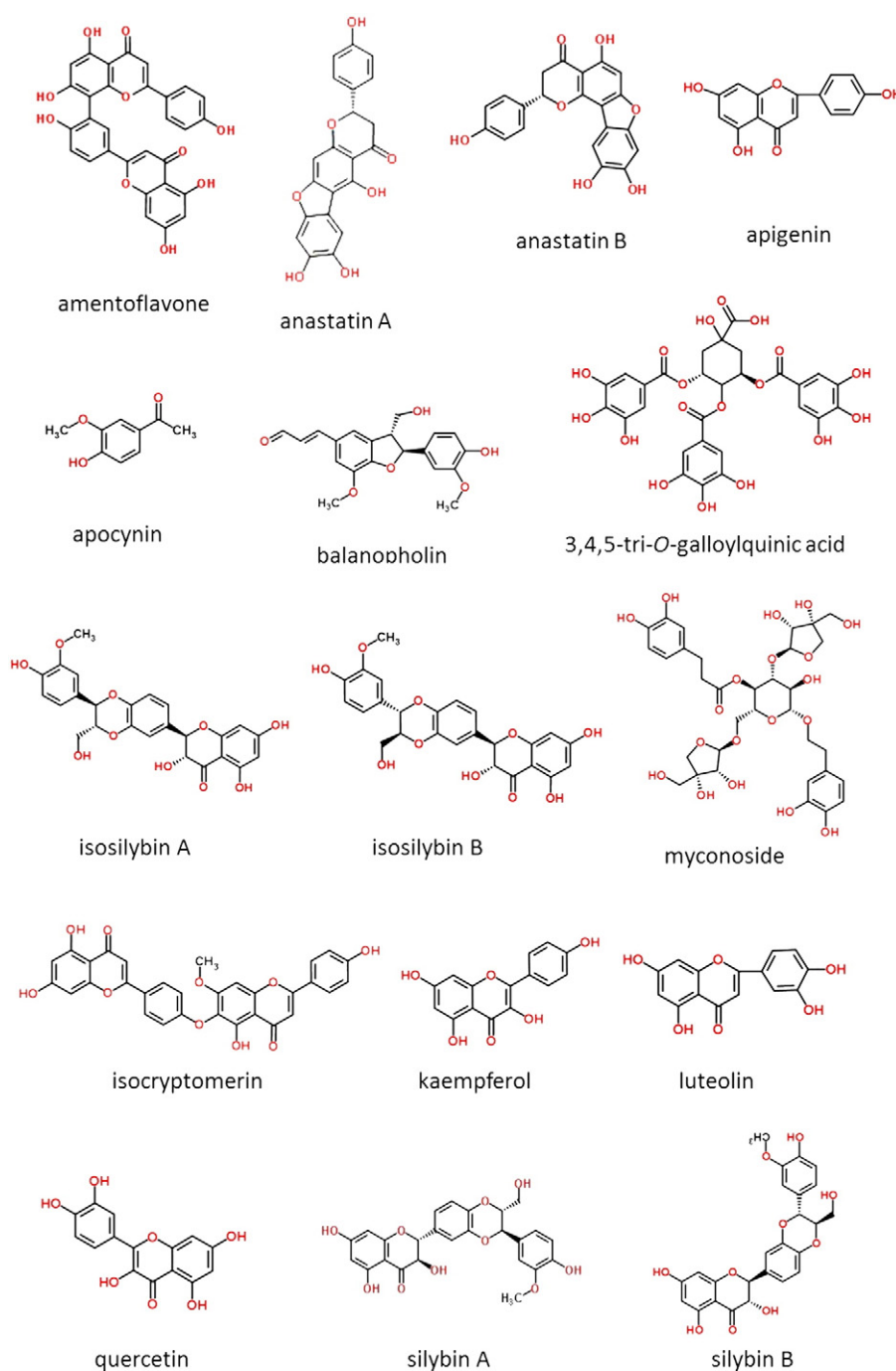


Fig. 2. Chemical compounds from resurrection plants with pronounced biological activities. Amentoflavone (*Selaginella tamariscina*); anastatins A and B (*Anastatica hierochuntica*); apigenin (*Anastatica hierochuntica*, *Selaginella tamariscina*); apocynin (*Boea hygrometrica*); balanopholin (*Anastatica hierochuntica*); 3,4,5-tri-O-galloylquinic acid (*Myrothamnus flabellifolia*); isocryptomerin (*Selaginella tamariscina*); isosilybin A (*Anastatica hierochuntica*); isosilybin B (*Anastatica hierochuntica*); kaempferol (*Anastatica hierochuntica*, *Haberlea rhodopensis*); luteolin (*Anastatica hierochuntica*, *Haberlea rhodopensis*); myconoside (*Haberlea rhodopensis*); quercetin (*Anastatica hierochuntica*, *Haberlea rhodopensis*); silybin A (*Anastatica hierochuntica*); and silybin B (*Anastatica hierochuntica*). For details about their (mode of) action and references, see Table 1. The structures and more details about their chemical properties are available at Pubchem (<http://pubchem.ncbi.nlm.nih.gov/>) and ChemSpider (<http://www.chemspider.com/>).

a tea or decoction) (Lohr et al., 2011; Moore et al., 2007). The biochemical composition of *M. flabellifolia* extracts depend on their geographical origins, which in turn reflect eco-physiological differences (Moore et al., 2005a). The major polyphenol in the Namibian population of *M. flabellifolia* was identified by NMR spectroscopy and confirmed by mass spectrometry to be 3,4,5-tri-O-galloylquinic acid (Moore et al., 2005b). It constitutes more than 90% of the total polyphenols and can accumulate to 44% or 74% by weight in hydrated and dehydrated leaves, respectively. 3,4,5-tri-O-galloylquinic acid was shown to protect linoleic acid against oxidation in vitro (Moore et al., 2005b); it stabilized an

artificial membrane system (liposomes) against desiccation. Based on the finding that the phase transition temperature of the liposomes was diminished by the presence of 3,4,5-tri-O-galloylquinic acid it was suggested that this polyphenol maintained the membranes in the liquid crystalline phase at physiological temperatures (Moore et al., 2005b). A polyphenol-rich extract of *M. flabellifolia* leaves showed inhibition to viral (M-MLV and HIV-1) reverse transcriptases, and the main active compound was 3,4,5-tri-O-galloylquinic acid. Inhibition of the two reverse transcriptases was non-competitive, with IC_{50} values of 5 μ M for M-MLV and 34 μ M for HIV-1 (Kamng'ona et al., 2011). The authors

Table 1

Activities of resurrection plants and relevant secondary metabolites in different models.

Resurrection species	Extract or pure compound	Biological effect	Reference
<i>Anastatica hierochuntica</i>	Anastatins A and B	Protection against D-galactosamine (D-GalN)-induced hepatotoxicity in mouse hepatocytes (IC ₅₀ 30 µM)	Yoshikawa et al., 2003
	Isosilybins A and B, luteolin, quercetin, (+)-balanophonin	Inhibition of melanogenesis in murine B16 melanoma 4A5 cells (IC ₅₀ values of 10–17 µM)	Nakashima et al., 2010
<i>Haberlea rhodopensis</i>	Myconoside-enriched fraction	Increases skin elasticity in humans (3% <i>Haberlea</i> extract creme) and stimulates elastin synthesis	Dell'Acqua and Schweikert, 2012
	Ethanol–water (70:30, v/v) extract	Protects human dermal fibroblasts against H ₂ O ₂ damage Dose-dependent reduction of chromosomal aberrations caused by γ-radiation in rabbit blood lymphocytes (120 mg kg ⁻¹ body weight)	Georgieva et al., 2013
<i>Myrothamnus flabelifolia</i>	3,4,5-tri-O-galloylquinic acid	Inhibits Moloney murine leukemia virus (M-MLV; IC ₅₀ 5 µM) and human immunodeficiency virus (HIV-1; IC ₅₀ 34 µM) reverse transcriptases	Kamng'ona et al., 2011
<i>Myrothamnus moschatus</i>	Acetone–water (70:30, v/v) extract	Activity against herpes simplex virus type 1 (HSV-1; IC ₅₀ 0.4 µg mL ⁻¹)	Gescher et al., 2011
	Essential oil (steam distillation for 3 h)	Antineoplastic activity: inhibits human breast cancer cells (MBA-MD-231; IC ₅₀ 15 µg mL ⁻¹) Antifungal activity against <i>Candida albicans</i>	Nicoletti et al., 2012
<i>Polypodium polypodioides</i> (<i>Pleopeltis polypodioides</i>)	Water extract (decoction or infusion)	Ethnopharmacological use in Americas as diuretic agent	Austin, 2004; Cano and Volpato, 2004
<i>Selaginella bryopteris</i>	Water extract	Promotes the growth of mouse macrophage (BMC2) and <i>Spodoptera frugiperda</i> Sf9 cells Protects Sf9 cells from UV-induced damage and H ₂ O ₂ -induced apoptosis (1–10% extracts) Reduces methyl isocyanate-induced apoptosis in human kidney epithelial cells (HEK-293) and human colon epithelial cells (FHC) Decreases incidents of dimethyl benzopyrene-induced lung carcinogenesis and benz[a]anthracene-mediated skin papillomagenesis in Swiss albino mice	Sah et al., 2005; Mishra et al., 2011
<i>Selaginella lepidophylla</i>	Methanol–water (10:90, v/v) extract	Activity against <i>Helicobacter pylori</i> (strains 43505 and 25), with MIC ₅₀ 200 and 400 µg mL ⁻¹ , respectively	Robles-Zepeda et al., 2011
<i>Selaginella tamariscina</i>	Water extract	Induces apoptosis in human leukemia cells (HL-60; cytotoxicity observed at 400 µg mL ⁻¹)	Ahn et al., 2006
	Ethanol–water (50:50, v/v) extracts	Antimetastatic activity in osteosarcoma cells (50 µg mL ⁻¹)	Yang et al., 2013a, 2013b
	Amentoflavone	Anti-proliferative and apoptotic effects against cervical cancer SiHa and CaSki cells (100 µM) Induces apoptosis in MCF-7 breast cancer cells and in <i>C. albicans</i> (10 µM) Antibacterial activity against <i>Staphylococcus aureus</i> (MIC ₅₀ 4 µg mL ⁻¹)	Lee et al., 2011; Pei et al., 2012; Hwang et al., 2012, 2013
<i>Tillandsia recurvata</i>	Isocryptomerin	Antimicrobial activity against <i>C. albicans</i> (MIC ₅₀ 18.11 µM) and the methicillin-resistant <i>Staphylococcus aureus</i> (MIC 10 µg mL ⁻¹)	Lee et al., 2009a, 2009b
	Cycloart-23-ene-3,25-diol-enriched extract	Inhibits several kinases associated with prostate cancer The closely related cycloartane-3,24,25-triol inhibits MRCKα kinase and shows high antineoplastic potential in prostate cancer cell lines (PC-3 and DU145; IC ₅₀ 2.23 and 1.67 µM, respectively)	Lowe et al., 2012a, 2012b

postulated that this compound, as well as related polymers, has the potential as an indigenous drug for antiviral therapy (Kamng'ona et al., 2011).

Acetone–water extracts from *M. flabellifolia* also exhibit strong activity against herpes simplex virus type 1 (HSV-1) (Gescher et al., 2011). The polyphenolic compounds of such extracts directly interact with viral particles, leading to oligomerization of the envelope proteins. Ultimately, the attachment of HSV-1 to the cell surface and entry into the cells are obstructed (Gescher et al., 2011).

Myrothamnus moschatus (Baillon) Niedenzu is another dicot resurrection shrub, similar to its relative *M. flabellifolia*, but endemic to Madagascar (Korte and Porembski, 2012). The major constituents of *M. moschatus* essential oil were *trans*-pinocarveol, pinocarvone, β -pinene, β -selinene, and perillyl acetate (Nicoletti et al., 2012). Traditionally, the dried leaves are smoked to treat asthma. Infusions of leaves (aqueous preparations) are used against cough and vomiting (Nicoletti et al., 2012). Essential oil of *M. moschatus* was found to inhibit the growth of human breast cancer cells (MBA-MD-231) with an IC_{50} of $15 \mu\text{g mL}^{-1}$. The oil also possesses antifungal activity, inhibiting the growth of *Candida albicans* (Nicoletti et al., 2012).

The resurrection fern **Pleopeltis polypodioides** (L.) E.G. Andrews & Windham (Polypodiaceae), also known as *Polypodium polypodioides*, is an epiphyte that grows on tree limbs and is widespread from southeastern US through most of Latin America (Austin, 2004; Layton et al., 2010). Various terpenoids belonging to the hopane, serratane, cycloartane, malabaricane and polypodane groups have been isolated from this fern (Ageta and Arai, 1990). *P. polypodioides* was used by the Aztecs as diuretic, against renal stones, cystitis and liver infections. The Houma uses a cold infusion to treat baby's sore mouths and applies a decoction to treat headache, bleeding gums, and dizziness. Other illnesses mentioned include bronchitis, hypertension, and fever (Austin, 2004).

Polypodium vulgare (L.) is another desiccation-tolerant fern that can also withstand salt and low temperature stresses (Bagniewska-Zadworna et al., 2008). It is very rich in phenolics and these compounds accumulate during desiccation and rehydration (Bagniewska-Zadworna et al., 2008). The species is also rich in phytoecdysteroids (Simon et al., 2011; Speranza, 2010). Interestingly, a number of phytoecdysteroids exerted promising reduction of multidrug resistance in L5178 mouse T-cell lymphoma cells (Martins et al., 2012).

The genus *Selaginella* (Selaginellaceae, Division Lycopodiophyta) is very rich in species (over 700), some of which are known resurrection plants and have been reported to possess medicinal properties (Swamy et al., 2006). The desiccation-tolerant *Selaginella bryopteris* (L.) is an Indian resurrection and medicinal plant and is very rich in flavonoids (Pandey et al., 2010; Swamy et al., 2006). Aqueous extracts of *S. bryopteris*, obtained from dried material, promoted the growth of mammalian mouse macrophage (BMC2) and *Spodoptera frugiperda* Sf9 cells (Sah et al., 2005). In addition, the *S. bryopteris* extract protected Sf9 cells from UV-induced damage and H_2O_2 -induced apoptosis (Sah et al., 2005). In another study, a flavonoid-rich benzene fraction of an aqueous extract from the same species was able to significantly reduce methyl isocyanate-induced apoptosis in human kidney epithelial cells (HEK-293) and human colon epithelial cells (FHC). In Swiss albino mice, the *Selaginella* extract decreased the incidents of dimethyl benzopyrene-induced lung carcinogenesis and decreased the number, size, and weight of benz[a]anthracene-mediated skin papillomagenesis (Mishra et al., 2011).

The spikemoss *Selaginella lepidophylla* (Hook. & Grev.) Spring (Selaginellaceae) inhabiting North America is a model resurrection plant with well-studied primary and secondary metabolites (Yobi et al., 2012; Yobi et al., 2013). It is commonly known as The False Rose of Jericho, Dinosaur Plant or doradilla and is easily confused with *A. hierochuntica*. *S. lepidophylla* is used in the form of a tea to treat colds and sore throat (Curtis and Moore, 1997). The inhibition of *Helicobacter pylori*, a human pathogen associated with gastric cancer,

by methanolic extracts obtained from dried *S. lepidophylla* plants indicates the potential of this species in medicine (Robles-Zepeda et al., 2011). Jericine is a commercially available aqueous extract from *S. lepidophylla* and recommended for anti-aging and regenerative moisturizing creams.

Its Chinese relative *Selaginella tamariscina* (P.Beauv.) Spring, also a resurrection plant (Wang et al., 2010), has attracted much more attention with its antibacterial, anti-inflammation, and anticancer (cytotoxic) activities. Extracts from dried *S. tamariscina* can induce apoptosis in human leukemia HL-60 cells (Ahn et al., 2006). Apoptosis was dependent on caspase-3 activation and production of reactive oxygen species, as addition of catalase or superoxide dismutase abolished apoptosis (Ahn et al., 2006).

Three novel sterols isolated from *S. tamariscina* were shown to inhibit growth of HL-60 cells (Gao et al., 2007). More recently, *S. tamariscina* extracts were found to suppress metastasis of human osteosarcoma cells (Yang et al., 2013a, 2013b), to induce apoptosis in both human cervical cancer cells and MCF-7 human breast cancer cells (Lee et al., 2011; Pei et al., 2012), and to attenuate metastasis of human oral squamous-cell carcinoma (Yang et al., 2013a, 2013b) (Table 1). Apoptosis was induced by the biflavonoid amentoflavone via a mitochondria-dependent pathway (Lee et al., 2011; Pei et al., 2012). Amentoflavone also induced apoptosis in *C. albicans* via a mitochondrial pathway (Hwang et al., 2012) and had an antibacterial effect, acting synergistically with antibiotics (Hwang et al., 2013). Pure amentoflavone was as active against *Staphylococcus aureus* as ampicillin, cefotaxime, and chloramphenicol (minimum inhibitory concentration, MIC $4 \mu\text{g mL}^{-1}$) (Hwang et al., 2013).

A novel biflavonoid from *S. tamariscina* called isocryptomerin exhibited antifungal and antibacterial activities on human pathogens with no hemolytic effects against human erythrocytes (Lee et al., 2009a; Lee et al., 2009b) (Table 1).

The monocot *Tillandsia recurvata* (L.) (Bromeliaceae), known as the Jamaican Ball Moss, is an American epiphyte that can withstand dehydration to below 30% relative water content and then hydrate again, depending on atmospheric conditions (Bermudez and Pignata, 2011). *T. recurvata* extract obtained from air-dried, pulverized plant material was shown to selectively inhibit a number of protein kinases known to be associated with prostate cancer (Lowe et al., 2012a; Lowe et al., 2012b). Using a competition binding assay, 451 protein kinases were tested and selective inhibition of five of them was reported (Lowe et al., 2012a). Two of the most inhibited, mitogen-activated protein kinase kinase 5 (MEK5) and cyclin G-associated kinase (GAK), are known to be associated with prostate cancer. The *T. recurvata* extract is rich in the cycloartane cycloart-23-ene-3,25-diol (Lowe et al., 2012b). The closely related triterpenoid cycloartane-3,24,25-triol inhibited the MRCK α kinase, which is associated with prostate cancer, and reduced the viability of the prostate cancer cell lines PC-3 and DU145 (IC_{50} values of 2.23 and $1.67 \mu\text{M}$, respectively) (Lowe et al., 2012b).

Genetic and genomic resources

The nuclear genome of *Physcomitrella patens* (480 Mbp, 35,938 protein-coding genes), a desiccation-tolerant moss, was first sequenced in 2008 and then re-annotated in 2013 (Rensing et al., 2008; Zimmer et al., 2013). The data provide an excellent resource for functional and comparative genomics. *P. patens* is a plant with efficient homologous recombination and a large number of knockout mosses are available through the International Moss Stock Center (<http://www.moss-stock-center.org/>). The genome of *Selaginella moellendorffii* (213 Mbp, 22,285 protein-coding genes), a desiccation-sensitive spikemoss closely related to the desiccation-tolerant species *S. lepidophylla*, was sequenced in 2011 (Banks et al., 2011). Additionally, mitochondria and/or chloroplast genomes of several other resurrection species have been sequenced (Oliver et al., 2010; Zhang et al., 2013). With the

advances of the next generation sequencing technologies (Thudi et al., 2012), new genome sequences are just a few steps away. Nevertheless, sequencing of resurrection genomes may not always be without complications, as some have large sizes and the lack of detailed genetic maps and the presence of repetitive sequences may render straightforward annotations difficult (Zonneveld et al., 2005).

Comprehensive transcriptome analysis using different technologies (RNA-seq, microarrays, ESTs) has been performed on a number of resurrection species in the past, including *P. patens* (both sporophyte and gametophyte), *Tortula ruralis*, *C. plantagineum*, *H. rhodopensis* (Collett et al., 2004; Gechev et al., 2013; Iturriaga et al., 2006; O'Donoghue et al., 2013; Oliver et al., 2004; Rodriguez et al., 2010; Xiao et al., 2011; reviewed by Dinakar and Bartels, 2013). In addition, the proteome of various resurrection plants was analyzed, including *S. tamariscina*, *B. hygrometrica*, *S. stapfianus*, and *Xerophyta viscosa* (Ingle et al., 2007; Jiang et al., 2007; Oliver et al., 2011b; Wang et al., 2010). These 'omics' approaches shed light not only on the mechanisms of desiccation tolerance, but also on aspects of plant development, and provided a better understanding of genes and proteins related to metabolism.

In parallel, systems for gene transfer/plant transformation and regeneration are rapidly being developed for resurrection species, and a few of them are already transformable (Strotbek et al., 2013), which is an essential step towards functional genomics and metabolite engineering.

Prospects of engineering secondary metabolite spectra in resurrection plants and establishing secondary metabolite pathways in microorganisms

Metabolic engineering has been very successful in model and crop plants (Butelli et al., 2008; Dixon et al., 2013; Haslam et al., 2013; Yamada and Sato, 2013) and is now also being developed for genetic engineering of secondary metabolite production in medicinal plants (Higashi and Saito, 2013). However, the pathways and enzymes for secondary metabolite production in resurrection species are often not well characterized yet. Furthermore, stable transformation is currently available for only a few resurrection plants, although given the increasing interest in such species the situation may change rapidly. An additional current limitation is that only few promoters (tissue- or organ-specific) have so far been characterized in detail in resurrection plants. The availability of such regulatory elements will be needed for proper control of secondary metabolite genes in genetic engineering approaches. However, there are numerous promoters known from other plants that can be tested for their expression behavior in resurrection species (Hieno et al., 2014; Peremarti et al., 2010; Porto et al., 2014; Shahmuradov et al., 2003) and computer-aided design tools may help to facilitate the development of new synthetic promoters (Mehrotra et al., 2011; Nishikata et al., 2014; Venter, 2007).

Even though genes for secondary metabolite biosynthesis pathways may not be known in detail, enhancing the production of such compounds in resurrection plants may be feasible. In particular, transcription factors (TFs) could be valuable tools to modify secondary metabolite spectra. Higher plants have ~2,000 different TFs (e.g., Pérez-Rodríguez et al., 2010; <http://plntfdb.bio.uni-potsdam.de/v3.0/>) and the developmental and physiological functions of many of them were unraveled over the last two decades. Modified levels of anthocyanins, alkaloids, and other secondary metabolites by altering TF gene expression have already been achieved in model and crop plants (Butelli et al., 2008; Dixon et al., 2013; Haslam et al., 2013; Higashi and Saito, 2013; Yamada and Sato, 2013), and TFs from the resurrection species *B. hygrometrica* and *C. plantagineum* have been used to alter metabolism and stress tolerance (Deng et al., 2006; Villalobos et al., 2004; Zhu et al., 2009). As TFs exert their control on metabolite spectra by regulating the expression of target genes, it will also be possible to identify such genes through global transcriptome analyses. For example, the TF can be transiently expressed in protoplasts prepared from resurrection plants,

followed by transcriptome profiling using RNA-seq or other global detection methods (e.g., microarray-based expression analysis). The general principle of such an approach has for example been demonstrated for *Arabidopsis thaliana* (Bargmann et al., 2013). Furthermore, computational methods have recently been developed to facilitate the annotation of transcripts even in the absence of whole-genome information (which is currently the case of many resurrection species) (Grabherr et al., 2011; Schulz et al., 2012). RNA-seq applied to resurrection species transiently transformed with homologous or heterologous TFs may thus assist in the identification of enzyme-encoding genes relevant for secondary metabolite biosynthesis.

Another important source of information that can be harnessed for metabolite engineering in resurrection plants is natural variation expected to exist between different populations, ecotypes, and sister species. There are sister species of desiccation-tolerant and desiccation-sensitive plants; for example, *Selaginella lepidophylla*, *Sporobolus stapfianus*, and *Lindernia brevidens* are desiccation-tolerant, while *Selaginella moellendorffii*, *Sporobolus pyramidalis*, and *Lindernia subracemosa* are desiccation-sensitive (Oliver et al., 2011a, 2011b; Van den Dries et al., 2011; Yobi et al., 2012). Furthermore, metabolite spectra can vary within a single species due to genetic variation between different populations/ecotypes and/or different growth conditions in the different ecological areals. For example, the Namibian and South African populations of *M. flabellifolia* differ in the compositions of their polyphenols and essential oils (Moore et al., 2005a). The resurrection fern *Mohria caffrorum* is desiccation-tolerant in the dry season and desiccation-sensitive in the rainy season (Farrant et al., 2009). The desiccation tolerance correlates with higher levels of galactinol, sugars like raffinose and melezitose, as well as higher activities of antioxidant enzymes such as catalases, glutathione reductases, and superoxide dismutases (Farrant et al., 2009). Recent years have shown an explosion in research with respect to natural variation within species. Particularly striking has been work on *A. thaliana* which among others has allowed the characterization of variance in flowering time, cold tolerance, seed dormancy (Alonso-Blanco and Koornneef, 2000; Koornneef et al., 2004) and more recently important information regarding metabolic regulation and its relation to ecological niches has been obtained (Kleessen et al., 2012; Sulpice et al., 2009; Sulpice et al., 2010). Such approaches have also been useful in crops such as rice (*Oryza sativa*), maize (*Zea mays*) and the Solanaceous species potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) where the focus has been on identifying the genetic basis of agronomic traits (Harjes et al., 2008; Kloosterman et al., 2013; Schauer et al., 2006; Xue et al., 2008). Knowledge about bioactive compounds present in specific ecotypes of resurrection plants or closely related species will further assist in identifying the genetic basis of secondary metabolite accumulation which can then be employed in genetic engineering approaches to optimize the yield of medicinally important compounds.

A further attractive approach towards utilizing genetic resources from resurrection plants involves strategies from the wide area of synthetic biology to establish partial or entire secondary metabolite pathways in microorganisms. Synthetic biological strategies have already successfully been employed to produce high titers of complex secondary metabolites such as artemisinic acid (or its precursor amorpha-4,11-diene) in *Escherichia coli* or the yeast *Saccharomyces cerevisiae* (reviewed in Keasling, 2012); other plant natural products like alkaloids were also produced in microbial organisms (Marienhagen and Bott, 2013). A major technical hurdle with respect to the engineering of secondary metabolite pathways lies in the fact that – as a rule – multiple genes must be assembled on functional genetic constructs (plasmids, synthetic chromosomes, modified natural chromosomes) to provide the genetic information required for all enzymes of a biosynthesis pathway.

Another important aspect to be considered when building multigene constructs for complex biosynthesis pathways is the fine-tuning of gene expression. Thus, getting the right promoters and cis-regulatory

elements stitched to the different enzyme-coding fragments is a critical issue and considerable effort is generally needed to find the optimal combination of regulatory and protein-coding DNA fragments (Blazek et al., 2012; Dehli et al., 2012; Siegl et al., 2013). Notably, synthetic TFs like custom-made transcription activator-like effectors (TALEs) or transcriptional regulators based on clustered regularly interspaced short palindromic repeats (CRISPR) offer interesting additional customizable tools for the precise control of gene expression on synthetic multigene constructs (Gilbert et al., 2013; Mercer et al., 2013).

Conclusions and outlook

Genome and transcriptome information gives an opportunity to study the pathways and identify some of the genes involved in the synthesis of metabolites with biotechnological importance. The sequencing of the nuclear and chloroplast genomes of *P. patens*, the chloroplast genome of *T. ruralis*, and the chloroplast and mitochondrial genomes of *B. hygrometrica*, provide good resources for comparative genomics and insights into the function and evolution of organelles in resurrection species (Oliver et al., 2010; Zhang et al., 2013). Similarly, transcriptome analyses of *H. rhodopensis* and *C. plantagineum* disclosed most of the genes present and differentially expressed in the two resurrection plants (Gechev et al., 2013; Rodriguez et al., 2010). Furthermore, genome information, combined with comprehensive metabolome profiling, can be used to identify important genes, metabolites, and pathways with roles in abiotic stress tolerance or potential biomedical applications (Saito, 2013; Tohge and Fernie, 2010). The potential of *P. patens* in this respect has already been highlighted (Decker and Reski, 2008).

The resurrection plants discussed here show health-promoting effects and in a number of cases this has been linked to particular secondary metabolites. Considering the apparent role of secondary metabolites for desiccation tolerance in resurrection plants, it is not surprising that antioxidant, cytotoxic, antibacterial, antifungal and antiviral properties have been found as the main biological activities of extracts and isolated constituents (often phenolic compounds). With the preclinical pharmacological research conducted so far, the traditional (ethnopharmacological) use of certain species is scientifically supported to some extent and promising results have already been obtained. However, in most of the studies extracts instead of purified substances have been analyzed. In these cases, the bioactive compounds need to be identified and their mode of action analyzed. Furthermore, potential side effects need to be investigated. In particular, in the case of anticancer (tumorcidal) activity, potential cytotoxic effects on other cell types must be studied to evaluate to what extent the compound is specific to tumor cells. Thus, more work is still to be done to develop interesting resurrection plant compounds into leads for potential novel drug substances. For production purposes, sustainable cultivation of resurrection plants may be a problem. Biomass accumulation is often limited and most species are difficult to cultivate and propagate. However, the establishment of cell or tissue culture systems from resurrections plants may be considered as a possible solution. In addition, a more profound knowledge of the regulation of biosynthetic pathways in these species may render possibilities for genetic approaches to develop feasible production systems. Finally, synthetic biology strategies offer opportunities to reconstruct entire or partial metabolite biosynthesis pathways from resurrection plants in microbial cell factors without harming natural populations of such species.

From the few studies highlighted here, it is clear that there is great potential in using resurrection plants for biomedical research and drug discovery. Given the number of resurrection plants known to date (estimated to be more than 1300; Porembski, 2011), the diverse ecosystems they inhabit, and the wide spectrum of secondary metabolites they presumably produce to adapt to these often harsh environments, we can conclude that only a small part of this rich species diversity has been explored in terms of metabolite composition and potential for

medical application. With recent technical advances in metabolomics, more detailed studies on secondary metabolites need to be performed and further compounds of relevance to medicine identified. The biological effect of these compounds on important human and animal pathogens, as well as on cancer cells must be evaluated in detail. The potential of resurrection plants can be further realized with the help of the accumulating genetic resources, the rapidly developing genomics technologies and the promising achievements in synthetic biology. Ultimately, engineering the pathways for their synthesis using genetic information gleaned from the genome and transcriptome analyses will likely result in the generation of high levels of natural products with desirable biological activities.

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